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KARYOTYPE VARIATION BY WHOLE ARM TRANSLOCATION IN ITALIAN SPECIMENS OF THE VICIA CRACCA GROUP (FABACEAE) (**)

Riassunto — *Variabilità cariotipica di campioni italiani del gruppo di Vicia cracca (Fabaceae), per traslocazione di bracci cromosomici.* Indagini precedenti evidenziano variabilità di numero cromosomico in alcuni membri del gruppo di *Vicia cracca*; tuttavia, almeno in Europa, per *Vicia tenuifolia*, risulta costantemente segnalato il numero $2n=24$. Nell'Italia nordoccidentale sono state ritrovate popolazioni di queste specie con numeri cromosomici nuovi, maggiori di $2n=24$, e talora variabili all'interno di una singola popolazione. Questi dati, confrontati con osservazioni precedenti, farebbero supporre che l'evoluzione cariotipica, in questo gruppo, possa avvenire per fusione e dissociazione centrica di cromosomi.

Abstract — Although previous studies point out karyotypic instability in other members of the *Vicia cracca* group, European data quote chromosome number uniformity for *Vicia tenuifolia*. New chromosome numbers and even intrapopulation chromosome number variability, in Italian populations of this species, are reported here. These data, together with a re-examination of former studies on this group, suggest that karyotype evolution, in the whole group, may occur by centric fusion and dissociation of chromosomes.

Key words — *Vicia cracca* — karyotype evolution — Robertsonian translocations (centric fusions/fissions).

Introduction

The genus *Vicia* is widley distributed throughout the temperate zones of both hemispheres, and has its major centre of variability in the Mediterranean Area (Hanelt and Mettin, 1970).

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It includes about 150-200 species (MELCHIOR, 1964) grouped, according to BALL (1968), into four sections. The basic number is stable (x=7) in only one of the sections (section *Ervum*), while it varies from 5 or 6 to 7 in all the others. In the *Cracca* section, represented in Italy by 22 entities (BALL, 1968), the different interspecific chromosome numbers reported up to now vary from $2n=10$ to $2n=28$: *V. serinica* has shown $2n=10$; *V. ochroleuca* and *V. cassubica* have been found to have $2n=12$; *V. sicula, V. sylvatica, V. onobrychioides, V. altissima* and *V. benghalensis* have shown $2n=14$. To this section belong two very critical groups, the *V. cracca* and the *V. villosa* aggregates, in both of which tetraploid cytotypes may be found (ROTI-MICHELOZZI, 1984, 1986).

Most of data from literature (BALL, 1968; MOORE, R.J., 1973, 1974, 1977; MOORE, D.M., 1982; GOLDBLATT, 1981, 1984, 1985, 1988; GOLDBLATT and JOHNSON, 1990) report for the species of the group the following chromosome numbers: in *V. cracca* $2n=14$, 27, 28, 30; in *V. incana* and in *V. dalmatica* $2n=12$, in *V. tenuifolia* $2n=24$. However several other chromosome numbers have been found in these taxa. *V. cracca* has also shown $2n=21$ (ROUSI, 1973); *V. incana* has been found to have $2n=13$ (RAINA and REES, 1983), *V. tenuifolia* has been found to have $2n=12$ by STANKEVITS, in Caucasia (1963, in ROUSI, 1973) and by YAMAMOTO (1973) in Japan, and even $2n=28$, in Iraq by AL-MAYAH and AL-SHEBAZ (1977). Clearly the chromosome numbers in this group are variable.

ROUSI (1961) originally observed the three chromosome numbers $2n=12$, 14 and 28, in populations of *V. cracca* from different localities; he supposed that the karyotype of *V. cracca* with $2n=12$ was derived from another karyotype of the same species with $2n=14$ through centric fusion of small submeta- or subtelocentric chromosomes (terminology according to LEVAN *et alii*, 1964), and that the karyotype of *V tenuifolia* ($2n=24$) was subsequently derived by duplication of the karyotype with $2n=12$ chromosomes. Later (1973) ROUSI recognized that the cytodemes formerly thought by him to belong to *V. cracca*, belonged instead to *V. incana*. All these karyotypes, however, were found in different populations. ROTI-MICHELOZZI (1984), on the contrary, noticed both chromosome numbers, 12 and 14, in one single population, showing the morphological features of *V. incana*. This entity, however, is extremely similar to *V. cracca*. The characters by which it may be distinguished from *V. cracca*, in fact, are mainly the different length ratio of the calyx tube to the calyx teeth, the lesser height and the greater hairiness. These features seem

to be very variable, and the last two mostly due to exposure to stronger sun in some localities. Therefore the three cytodemes of *V. incana* examined by ROTI-MICHELOZZI (1984) were considered to be separate from *V. cracca* only at infraspecific level, and consequently thought to belong to *V. cracca* L. ssp. *gerardii* Gaudin, in agreement with DAVIS (1970). This opinion, though with a different nomenclature, was shared by GREUTER *et alii* (1989). However, for convenience, in this paper the nomenclature given by BALL (1968) will be followed.

Moreover, intraclonal chromosome variation between $2n=14$ and 28, including odd chromosome numbers (mixoploidy), has been described in *V. cracca* sensu stricto (ROUSI, 1961; CHRTKOVA-ZERTOVA, 1973; ROTI-MICHELOZZI, 1984).

In this paper new reports on intraspecific variation of chromosomes in the *V. cracca* group are presented and discussed in relation to karyotypic diversification and evolutionary mechanisms.

MATERIAL AND METHODS

The material, including material previously studied by ROTI-MICHELOZZI (1984), consisted of specimens of *V. cracca, V. incana* and *V. tenuifolia*, collected from natural populations in several Northwestern Italian localities, listed in Table 1. Voucher dry specimens from these populations were deposited at the Genova Herbarium (GE).

For the karyological studies, actively growing healthy root tips were harvested from seeds, germinated in Petri dishes on moist sterilized paper filters. They were pretreated either with an 0.2-0.4% aqueous solution of colchicine for 2 hours or with an 0.2% aqueous solution of glucose at 0° overnight (12 hrs), fixed in aceto-alcohol (1/3), hydrolysed with HC1 1N at 60° for 6-10′, stained with 1-1.5% Gomori's hematoxylin (MELANDER and WINGSTRAND, 1953), and squashed onto permanent slides, vouchers of which are kept in the Genova Botanic Institute. At least five, but generally about ten well-clarified metaphase plates were photographed for each population. For the karyometric analysis, the negatives thus obtained, except for one population in which the chromosomes could only be counted, were projected using a 35 mm slide projector on drawing paper at a given magnification and sketched. The chromosome lengths were recorded in millimeters and converted into micrometers, in order to produce the karyograms. In the karyograms the chromosomes

TABLE 1 - *Origins, chromosome numbers and karyotypic formulae of the studied material.*

Species	Origin	2*n*	Karyotypic formula
Vicia cracca	Rezzoaglio (Genova Province)	14	10sm+4st
	S. Pietro d'Olba (Savona Province)	14	10m+4sm
	Verrand, Prè S. Didier (Aosta Province)	28	2m+20sm+6st
	Larizzate (Vercelli Province)	14, 15, 17, 22....28	2m+20sm+6st (only for 2*n*=28)
V. incana	Near Rapallo (Genova Province)	12, 14	2Lm+8sm+2st 12sm+2st
	S. Stefano d'Aveto (Genova Province)	12	2Lm+8sm+2st
	La Carta (Savona Province)	12	2Lm+10sm
V. tenuifolia	Pallusieux (Aosta Province)	24	4Lm+10sm+10st
		25	3Lm+2m+12sm+8st
		26	2Lm+14sm+10st
		27	1Lm+2m+12sm+12st
		28	26sm+2st
	Chabodey (Aosta Province)	24, 25, 26, 27, 28	
	S. Giacomo di Roburent (Cuneo Province)	28	2m+16sm+10st
	Quarto Alto (Genova Province)	24	4Lm+12sm+8st
		25	3Lm+14sm+8st
		26	2Lm+16sm+8st
		27	1Lm+12sm+14st
		28	2m+12sm+14st

were arranged in order of decreasing length. The karyotypic formula, according to LEVAN *et alii*, 1964 (which has been slightly modified, as follows), was designated for each karyogram obtained. In the karyotypic formulae the chromosome were indicated as «Lm», if large metacentrics, as «m» if small metacentrics, as «sm» if submetacentrics and «st» if subtelocentrics. No telecentrics were found. The class of the submetacentric chromosomes included chromosomes with an arm ratio ranging between 1.6 and 3. For convenience, in the formulae, the

chromosomes with similar arm ratios were grouped together and not listed in order of decreasing length, and therefore the satellited couple was not pointed out.

Results

The material previously studied (Roti-Michelozzi, 1984) had shown, in the *V. cracca* populations, diploidy ($2n=14$) in the Ligurian localities, tetraploidy in the Aosta Valley population ($2n=28$, fig. 1c), mixoploidy in the Piedmont Po Valley ($2n=14$, 17, 22,...,28). Both in the diploid and in the tetraploid karyotypes the nucleolar constrictions were not always clearly visible and, when they were easily discernible, their position in the karyotypes was variable, thus indicating a possible structural polymorphism of these karyotypes.

The material which showed the morphological features of *V. incana* was found to have $2n=12$ in two populations and both $2n=12$ and 14 in a third population (Figs. 1a, 1b, Table 1). In this material no chromosome with nucleolar constriction was visible.

As for *V. tenuifolia*, after the first study on the population found in the Aosta Valley, which showed a karyotype with four large symmetric and twenty small asymmetric chromosomes and no discernible nucleolar organizer chromosome (Roti-Michelozzi, 1984), other wild specimens were subsequently found on the Maritime Alps and on the Ligurian Coast, as well as in another locality of the Aosta Valley (from these last ones only chromosome counts could be made). Since all the metaphase plates of the population from the Maritime Alps showed the chromosome number $2n=28$, a number not yet reported for material from Europe (Figs. 1d, 2f, Table 1), while the metaphase plates from the population of the Ligurian Coast showed chromosome numbers varying from $2n=24$ and 28 (Figs. 1e, 1f, 1g, Table 1), newly collected material from the first locality of the Aosta Valley was carefuly reexamined. This study revealed, also for this population, in a single as well as in different root tips, chromosome numbers variable from $2n=24$ to 28, and possibly a structural polymorphism due to presence-absence or different position of the nucleolar chromosomes (Figs. 2a, 2b, 2c, 2d, 2e, Table 1). Also the specimens of the other population found in the second locality of the Aosta valley similarly showed different chromosome numbers. Moreover, it was clear that the different chromosome numbers represented a disploid series, the number of metacentrics being inversely proportional to the total chromosome number, with no change in the total number of major chromosome arms. The

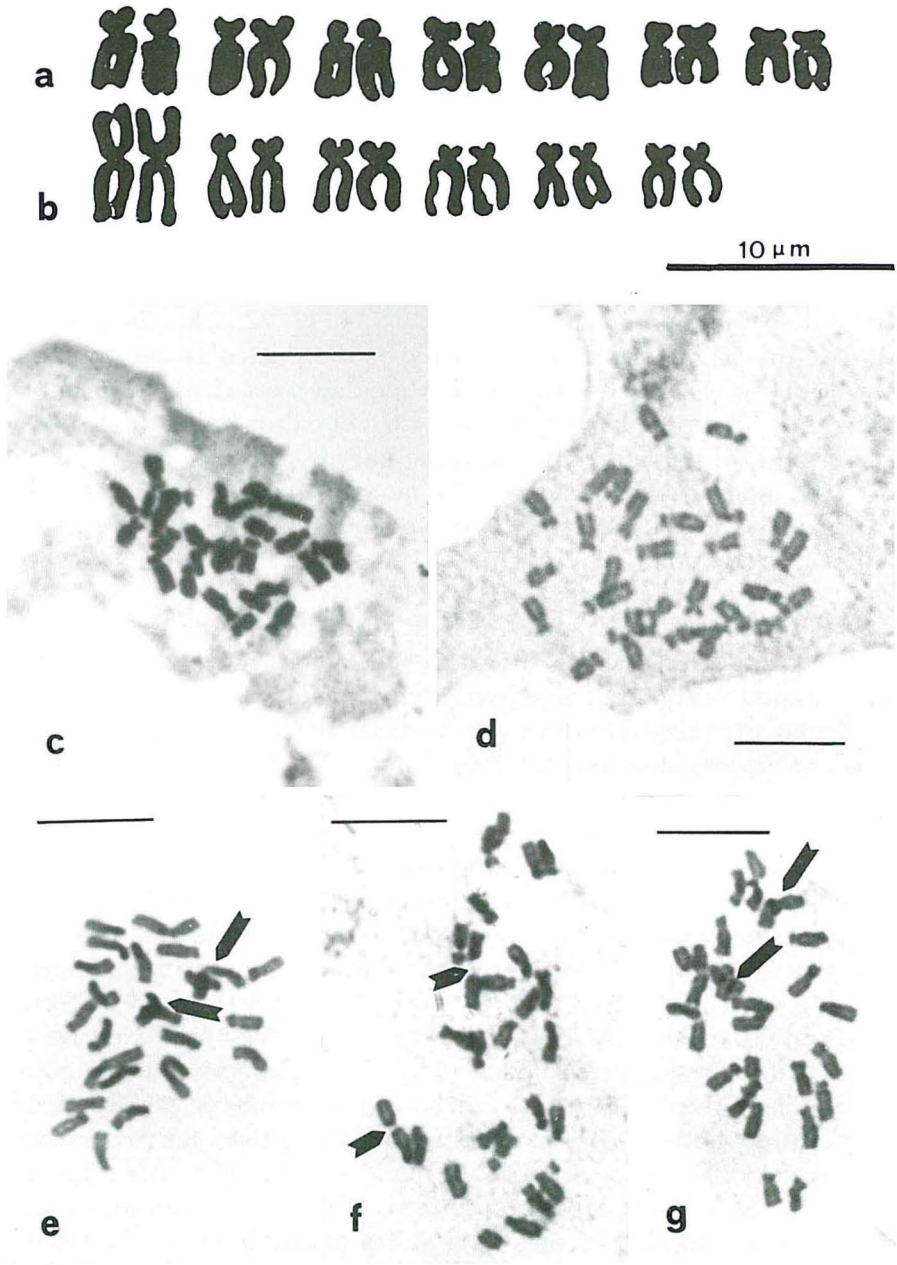

Fig. 1 - a-b Karyograms of *Vicia incana* from near Rapallo; a, $2n=14$; b, $2n=12$. c, Metaphase plate of the tetraploid strain of *Vicia cracca*. d-g. Metaphase plates of *Vicia tenuifolia*. d, S. Giacomo di Roburent population, $2n=28$. e-g, Quarto Alto population: e, $2n=24$; f, $2n=26$; g, $2n=28$. Bar: 10 µm. Long arrows indicate overlapping chromosomes, short arrows show chromosome arms separated at the centromere.

metaphase plates with 28 chromosomes were similar to those of the Maritime Alps population (Figs. 1d, 2e, 2f). It must be noted that all the mature legumes of the Aosta Valley and the Ligurian Coast populations were full with seeds and quite fertile. Therefore plants with different chromosome number were interfertile, or these different numbers could occur in the same plant; evidently certain couples of submeta or subteleconcentric chromosomes must be equivalent to larger metacentric chromosomes, and pair with them in meiosis, so that fertility is maintained.

Discussion and concluding remarks

As stated above, the involvement of centric fusions of chromosomes in the evolution of the *V. cracca* group was first claimed by Rousi (1961), with a trend towards a reduction of chromosome number, consistently with the views of Goldblatt (1979) for *Galaxia*, Norstog (1980) for *Zamia*, Jones (1978) for *Gibasis* and (1990) for *Tradescantia*. Therefore the evolutionary trend of this group would at first have been from diploid *V. cracca* ($2n=14$) towards diploid *V. incana* ($2n=12$) through centric fusion of chromosomes. According to Rousi (1973), this evolutionary step must have taken place at a very early stage in the evolution of the *V. cracca group*. Strains of *V. incana* could have originated, later, by polyploidization, strains of *V. tenuifolia*, with $2n=24$.

The finding of specimens of *V. tenuifolia* with different chromosome numbers, in the same population, both on the mild Ligurian Coast, and on the border of the highest Alpine Chain, and, at the same time, the finding of a population of this species on the lower Maritime Alps, with a stable but different and higher chromosome number, supports Rousi's hypothesis only to a certain extent. Moreover, the fact that the large metacentric chromosomes often break up at the centromere when these chromosomes are squashed (Figs. 1f, 2a, 2d) would account for a structural instability of centromeres in these chromosomes, such as has been noted in some chromosomes of a few *Zamia* species (Moretti, 1990). Structural instability of chromosomes had already been noticed in various populations of *V. cracca* sensu stricto (Roti-Michelozzi, 1984), populations which showed mixoploidy and/or presence-absence or different position of nucleolar constrictions. This last feature was also noticeable in the newly obtained karyograms of the Pallusieux (Aosta Valley)

population of *V. tenuifolia* (Fig. 2). According to BENNETT and GRIMSHAW (1991), in fact, the presence of different karyotypes in different cells within the same root, al least in *Cyclamen*, indicates a high degree of karyotype instability. The instability of the centromeres could be the cause of the subdivision of the large metacentric chromosomes of *V. tenuifolia* into two small subtelo- or submetacentric chromosomes each (centric fission) (Fig. 2). Therefore a reversal of trend could have originated, in this entity, an increasing chromo-

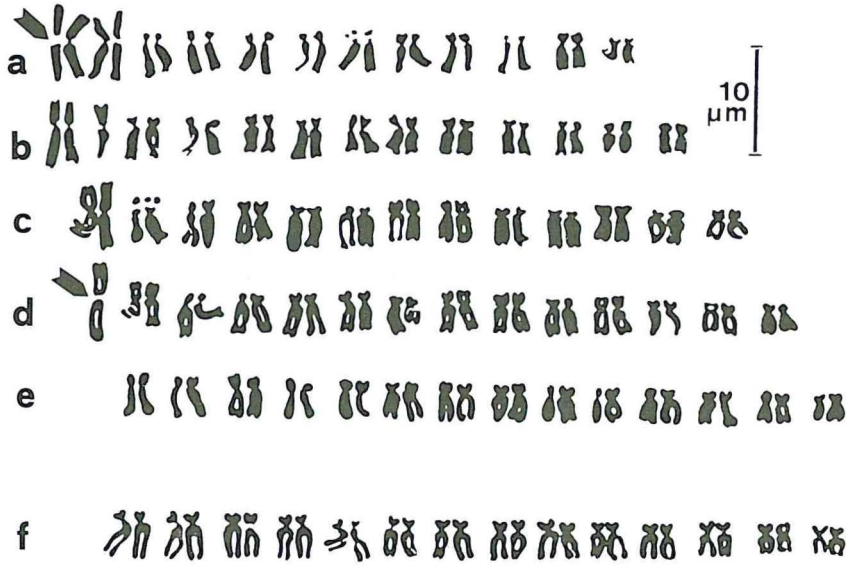

Fig. 2 - a-e. Karyograms of *Vicia tenuifolia* from Pallusieux. a, $2n=24$; b, $2n=25$; c, $2n=26$; d, $2n=27$; e, $2n=28$. f, Karyogram of *Vicia tenuifolia* from S. Giacomo di Roburent, $2n=28$. Bar: 10 µm. Short arrows point out chromosome arms separated at the centromere.

some number, from $2n=24$ to $2n=28$, by centric fission, the same trend as has been suggested by MORETTI and SABATO (1984) and MORETTI (1990) for some *Zamia* species. This supposition also derives from the fact that in all the other European localities the chromosome number of *V. tenuifolia* was stable and was $2n=24$, while the higher chromosome numbers were found only in the Italian populations. According to JONES (1978), in fact, when the relative frequency of the chromosome situation under consideration is sporadic, it is usually considered to be a derived state. Centric fission rather than centric fusion most likely would therefore be responsible for karyotypic variation in these populations.

In this way the whole *V. cracca* group could provide evidence that instead of being the outcome of an evolution occurred a long time ago, as has been supposed by Rousi (1973), it is still evolving. This hypothesis could explain the centric fusions still happening in the *V. incana* karyotypes and, on the other hand, the centric fissions in those of *V. tenuifolia*; it could also explain the numerous intergradations among the single taxa and the controversial chromosome numbers found.

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